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Article

Four New Jacaranone Analogs from the Fruits of a Beibu Gulf Mangrove *Avicennia marina*

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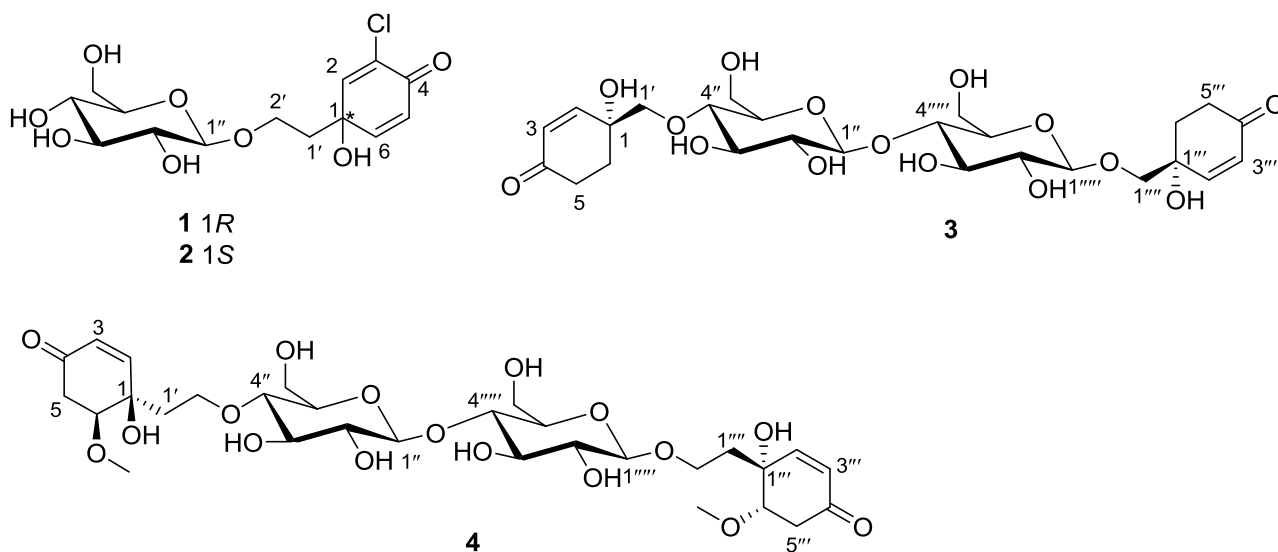
Abstract: Four new jacaranone analogs, marinoids F–I (**1–4**), were isolated from the fruits of a Beibu Gulf mangrove *Avicennia marina*. The structures were elucidated based on analysis of spectroscopic data. Marinoids F and G are shown to be diastereoisomers of chlorocornoside, a new halogen containing marine secondary metabolite. The antioxidant activity of the isolates was evaluated using a cellular antioxidant assay, and **4** showed good antioxidant activity ($EC_{50} = 26 \mu\text{M}$).

Keywords: antioxidant; *Avicennia marina*; chlorocornoside; cornoside; jacaranone analogs; marinoid

1. Introduction

Avicennia marina (Forsk.) Vierh. is commonly known as the grey or white mangrove plant resident in the tropical and subtropical regions, it is extremely widespread along the coasts of eastern Africa, islands of the Indian Ocean, tropical Asia, Australia, New Zealand, and islands of the Pacific Ocean to Fiji [1]. The crude extracts are reported to possess antimalarial and cytotoxic activities [1]. Different parts of the plant are used in Egypt as a folk medicine cure for skin diseases [2]. Previous chemical investigation of plants of the genus *Avicennina* have exhibited the presence of iridoid glucosides, marinoids A–E [1–5], naphthoquinone derivatives [6,7], flavonoids [4,8], and diterpenoids [9]. However, these previous studies did not report any chemical and biological data from the fruits of *A. marina*. Searching for bioactive secondary metabolites from this specimen afforded four new jacaranone analogs, marinoids F–I (1–4 respectively) (Figure 1). In this paper, we describe the isolation, structural elucidation, and antioxidant activity of the four new secondary metabolites.

Figure 1. Structures of marinoids F–I (1–4).



2. Results and Discussion

Marinoid F (1) was purified as a yellow oil with the molecular formula $C_{14}H_{19}ClO_8$ as determined by HRESIMS (found $[M + H]^+$ at m/z 351.0835, calcd $[M + H]^+$, 351.0841) as well as 1H and ^{13}C spectroscopic data (Table 1). 1H NMR spectra disclosed the presence of two methylene groups $[(\delta_H$ 3.94, ddd, $J = 10.1, 7.0, 2.2$ Hz, H-2' α) and 3.64, dd, $J = 10.1, 2.2$ Hz, H-2' β) and $(\delta_H$ 2.08, d, $J = 7.0$ Hz, H-1')], an α,β -unsaturated carbonyl group $[(UV \lambda_{max}$ 220 nm; δ_H 7.16 (d, $J = 2.8$ Hz, H-2); 6.99 (dd, $J = 10.0, 2.8$ Hz, H-6) and 6.22 (d, $J = 10.0$ Hz, H-5)]. The characteristic chemical shift of the carbonyl resonance (δ_C 179.3, C-4), in addition to the presence of four olefinic groups [δ_C 152.8 (C-6), 148.9 (C-2), 130.5 (C-3) and 125.8 (C-5)], and a quaternary sp^3 carbon (δ_C 70.2, C-1) (Table 1), demonstrated that 1 has a *para*-quinol-type partial structure [10,11]. NMR spectra also indicated the presence of a β -glucosyl group, *i.e.*, one anomeric carbon resonance at δ_C 102.6 (C-1'') and one anomeric proton at δ_H 4.18 (1H, d, $J = 9.2$ Hz, H-1''). It was used as a starting point in the homonuclear

correlated spectra to determine all glycosidic protons. The $J_{H-1''-H-2''}$ value (9.2 Hz) of compound **1**, further confirmed that the sugar was a β -glucosyl group [12].

Table 1. ^1H and ^{13}C NMR data of marinoids F (**1**) and G (**2**)^a.

Position	1		2	
	δ_{C} , Mult	δ_{H} (J in Hz)	δ_{C} , Mult	δ_{H} (J in Hz)
1	70.2, C		70.2, C	
2	148.9, CH	7.16 (d, 2.8)	148.6, CH	7.20 (d, 2.8)
3	130.5, C		130.0, C	
4	179.3, C		179.1, C	
5	125.8, CH	6.22 (d, 10.0)	125.4, CH	6.19 (d, 10.0)
6	152.8, CH	6.99 (dd, 10.0, 2.8)	153.1, CH	7.03 (dd, 10.0, 2.8)
1'	39.6, CH ₂	2.08 (dd, 11.2, 7.0)	39.7, CH ₂	2.07 (dd, 11.2, 7.0)
2' α	63.9, CH ₂	3.94 (ddd, 10.1, 7.0, 2.2)	64.0, CH ₂	4.00 (dt, 10.4, 7.0, 2.4)
β		3.64 (dd, 10.1, 2.2)		3.65 (dd, 10.4, 2.4)
1''	102.6, CH	4.18 (d, 9.2)	102.8, CH	4.21 (d, 7.8)
2''	73.6, CH	3.13 (9.6, 9.2)	73.6, CH	3.14 (dd, 9.6, 7.8)
3''	76.6, CH	3.20 (m)	76.6, CH	3.12 (m)
4''	70.1, CH	3.24 (m)	69.9, CH	3.24 (m)
5''	76.5, CH	3.29 (m)	76.6, CH	3.31 (m)
6'' α	61.3, CH ₂	3.81 (dd, 11.9, 2.0)	61.4, CH ₂	3.84 (d, 11.8)
β		3.62 (m)		3.65 (m)

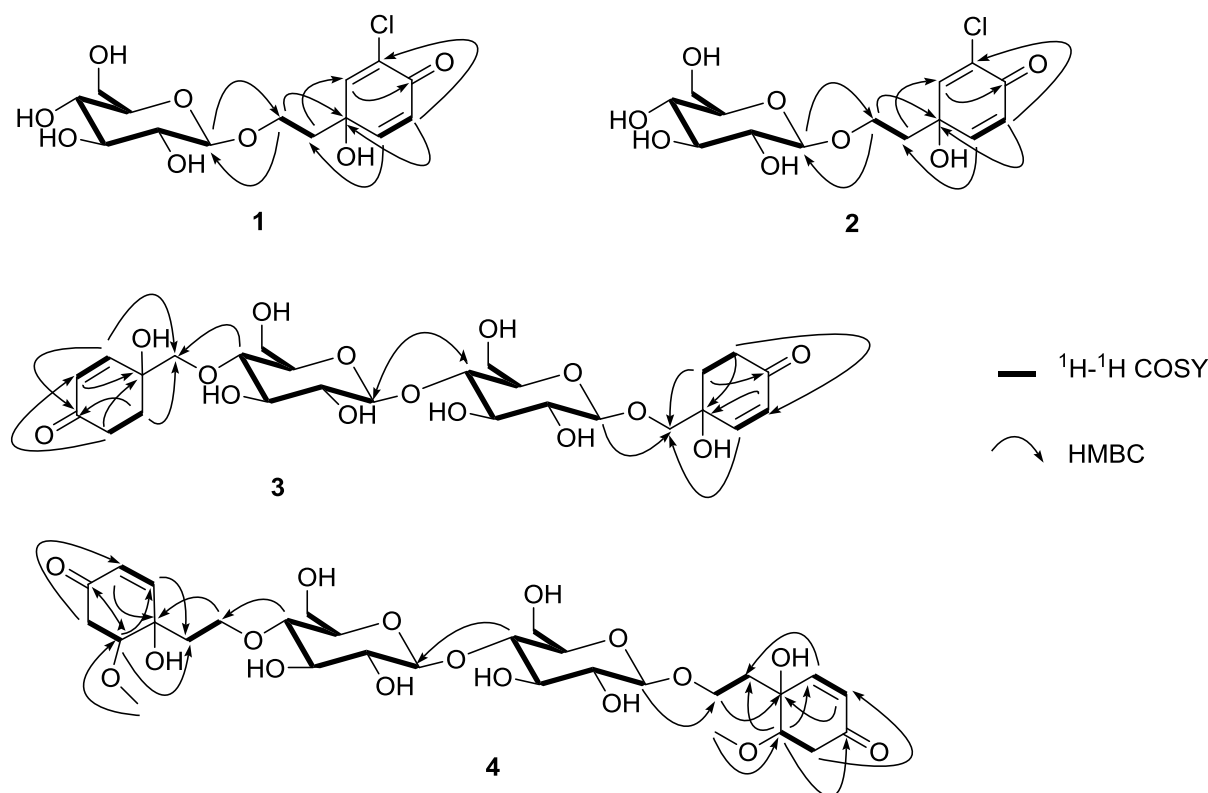
^a In CD₃OD, 600 MHz for ^1H and 150 MHz for ^{13}C NMR.

The gross structure was further established by the aid of COSY and HMBC experiments (Figure 2). A careful comparison of **1** and cornoside revealed that **1** differs from cornoside by the presence of one chlorine atom attached at C-3 [13]. Compound **1** showed $[\alpha]_{\text{D}}^{20}$ (MeOH) of -14.7° . The reported rotation value for cornoside is negative $[\alpha]_{\text{D}}^{20} -10.5^\circ$ [13], whose stereochemistry of the aglycone and the β -D-glucosyl residue have been established by enzymatic hydrolysis and other methods [13–15]. The reported rotation value after poly-acetylation of cornoside is also negative ($[\alpha]_{\text{D}}^{20} -10.2^\circ$) [16]. Moreover, the NMR data of the β -glucosyl residue in compound **1** are in accord with those observed in cornoside [13]. The substitution is simply that of one Cl atom for one H atom, and that many bonds away from the key chiral centre, therefore following those Literature data, we propose that the configuration of C-1 in compound **1** is the same as that found at C-1 in cornoside, namely *R*. Thus, the structure of **1** is predicted to be as shown in Figure 1.

Marinoid G (**2**) was obtained as yellow oil. Its molecular formula was determined as C₁₄H₁₉ClO₈ by HRESIMS (found $[\text{M} + \text{H}]^+$ at m/z 351.0837, calcd $[\text{M} + \text{H}]^+$, 351.0841) as well as ^1H and ^{13}C data (Table 1). The NMR spectra of **2** are very similar to those of **1**. In addition, analysis of the COSY, HMBC and NOSEY correlations of **2** revealed identical spin systems and connections with those found in **1**. Compound **2** showed $[\alpha]_{\text{D}}^{20}$ (MeOH) of $+10.2^\circ$, and the reported value for cornoside ($[\alpha]_{\text{D}}^{20} -10.5^\circ$) and poly-acetylated cornoside ($[\alpha]_{\text{D}}^{20} -10.2^\circ$) are both negative [13], and the observed value for **1** is -14.7° . Although we do not have an explanation for the differences in the absolute values of the optical rotations of cornoside, poly-acetylated of cornoside, and compound **1**, and the NMR data of the β -glucosyl residue in compound **2** are greatly similar to those of the β -glucosyl

residue in cornoside and compound **1**, and also compound **2** showed a positive Cotton effect at 220 nm ($\Delta\epsilon +5.97$), whereas the observed value for **1** was -4.28 , the opposite optical rotation and Cotton effect indicate that **1** and **2** are diastereoisomers. Indeed, we propose that they are enantiomers of the aglycone each with β -glucosyl residues.

Figure 2. Selected ^1H - ^1H COSY and HMBC correlations of marinoids F–I (**1**–**4**).



Marinoid H (**3**) was obtained as a colourless oil and its molecular formula was established as $\text{C}_{26}\text{H}_{38}\text{O}_{15}$ by HRESIMS (found $[\text{M} + \text{H}]^+$ at m/z 591.2281, calcd $[\text{M} + \text{H}]^+$, 591.2283) as well as ^1H and ^{13}C data (Table 2). In the NMR spectra of compound **3**, the proton signals at $[\delta_{\text{H}}$ 6.93 (1H, d, $J = 15.1$ Hz, H-2), 5.93 (1H, dd, $J = 15.1, 2.6$ Hz, H-3), 2.68 (1H, m, H-5 α), 2.49 (1H, m, H-5 β), 2.09 (1H, m, H-6 α), and 2.03 (1H, m, H-6 β)], and $[\delta_{\text{H}}$ 6.90 (1H, d, $J = 15.1$ Hz, H-2''), 5.92 (1H, dd, $J = 15.1, 2.6$ Hz, H-3''), 2.67 (2H, m, H-5''), 2.08 (1H, m, H-6'' α), and 2.01 (1H, m, H-6'' β)] and the carbon signals at $[\delta_{\text{C}}$ 199.1 (C-4), 152.8 (C-2), 128.1 (C-3), 70.9 (C-1), 42.1 (C-5), and 37.3 (C-6)] and $[\delta_{\text{C}}$ 199.0 (C-4''), 152.5 (C-2''), 127.8 (C-3''), 70.7 (C-1''), 42.0 (C-5''), and 37.2 (C-6'')] indicated the existence of two cyclohexanone moieties [17], which were further confirmed by HMBC connections. NMR spectra also indicated the presence of two β -glucosyl groups by two anomeric carbon resonances at δ_{C} 102.9 (C-1'') and 102.8 (C-1'''), and two anomeric protons at δ_{H} 4.27 (1H, d, $J = 7.8$ Hz, H-1'') and 4.25 (1H, d, $J = 7.8$ Hz, H-1'''), which were further confirmed by the $J_{\text{H-1}''\text{-H-2}''}$ and $J_{\text{H-1}'''\text{-H-2}'''}$ values [18]. The tentative molecular weight of the compound deduced from NMR analysis suggested compound **3** to be an unsymmetrical dimer.

Table 2. ^1H and ^{13}C NMR data of marinoids H (3) and I (4) ^a.

Position	3		4	
	δ_{C} , Mult	δ_{H} (J in Hz)	δ_{C} , Mult	δ_{H} (J in Hz)
1	70.9, C		72.4, C	
2	152.8, CH	6.93 (d, 15.1)	154.7, CH	6.95 (d, 10.0)
3	128.1, CH	5.93 (dd, 15.1, 2.6)	127.1, CH	5.87 (d, 10.0)
4	199.1, C		198.6, C	
5 α	42.1, CH ₂	2.68 (m)	38.9, CH ₂	2.82 (dd, 11.1, 3.6)
β		2.49 (m)		2.49 (dd, 11.1, 7.0)
6 α	37.3, CH ₂	2.09 (m)	82.8, CH	3.67 (dd, 7.0, 3.6)
β		2.03 (m)		
1' α	61.3, CH ₂	3.83 (d, 10.1)	34.4, CH ₂	2.18 (dd, 11.1, 5.3)
β		3.85 (d, 10.1)		2.00 (dd, 11.1, 6.1)
2' α			61.3, CH ₂	3.86 (d, 10.1)
β				3.79 (d, 10.1)
1''	102.9, CH	4.27 (d, 7.8)	103.0, CH	4.28 (d, 7.5)
2''	73.6, CH	3.13 (dd, 9.4, 7.8)	73.6, CH	3.14 (dd, 9.4, 7.8)
3''	70.9, CH	4.08 (m)	70.2, CH	3.26 (m)
4''	76.6, CH	3.32 (m)	76.7, CH	3.33 (m)
5''	76.6, CH	3.25 (m)	76.7, CH	3.26 (m)
6'' α	64.9, CH ₂	4.13 (dt, 9.5, 5.0)	65.1, CH ₂	4.16 (dt, 9.5, 5.0)
β		4.09 (dt, 9.5, 5.0)		4.14 (dt, 9.5, 5.0)
1'''	70.7, C		72.4, C	
2'''	152.5, CH	6.90 (d, 15.1)	154.0, CH	6.93 (d, 10.0)
3'''	127.8, CH	5.92 (dd, 15.1, 2.6)	127.0, CH	5.85 (d, 10.0)
4'''	199.0, C		198.6, C	
5''' α	42.0, CH ₂	2.67 (m)	38.9, CH ₂	2.82 (dd, 11.1, 3.6)
β				2.49 (dd, 11.1, 7.0)
6''' α	37.2, CH ₂	2.08 (m)	82.7, CH	3.64 (dd, 7.0, 3.6)
β		2.01 (m)		
1''' α	61.3, CH ₂	3.63 (d, 10.1)	34.3, CH ₂	2.17 (dd, 11.1, 5.3)
β		3.65 (d, 10.1)		1.98 (dd, 11.1, 6.1)
2''' α			61.3, CH ₂	3.84 (d, 10.1)
β				3.64 (d, 10.1)
1''''	102.8, CH	4.25 (d, 7.8)	102.8, CH	4.27 (d, 7.5)
2''''	73.6, CH	3.13 (dd, 9.4, 7.8)	73.6, CH	3.14 (dd, 9.4, 7.8)
3''''	70.2, CH	3.25 (m)	70.2, CH	3.24 (m)
4''''	76.6, CH	3.31 (m)	76.6, CH	3.31 (m)
5''''	76.6, CH	3.31 (m)	76.6, CH	3.24 (m)
6'''' α	64.9, CH ₂	3.78 (dt, 9.8, 5.5)	64.8, CH ₂	3.82 (dt, 9.8, 5.5)
β		3.78 (dt, 9.8, 5.5)		3.80 (dt, 9.8, 5.5)
OCH ₃			56.9, CH ₃	3.43 (s)
OCH ₃			56.9, CH ₃	3.43 (s)

^a In CD₃OD, 600 MHz for ^1H and 150 MHz for ^{13}C NMR.

The gross structure was further established by the aid of COSY and HMBC experiments (Figure 2). Six spin systems could be revealed by analysis of COSY correlations corresponding to the H-2/H-3, H₂-5/H₂-6, H-1''/H-2''/H-3''/H-4''/H-5''/H₂-6'', H-2'''/H-3''', H₂-5'''/H₂-6''', and H-1''''/H-2''''/H-3''''/H-4''''/H-5''''/H₂-6'''. The connectivity of the two cyclohexanone moieties to C-1' and C-1''' were established by the HMBC correlations of H-2 to C-1' and H-6 to C-1', and of H-2''' to C-1''' and H-6''' to C-1''', respectively. The presence of the two β-glucosyl groups in **3** could be proposed on the basis of HMBC correlations from H-4'' to C-1' and H-1'''' to C-1''', respectively. The connection of the two β-glucosyl groups was confirmed by the presence of HMBC correlation from H-4'''' to C-1''. The configuration of C-1 and C-1''' were assigned as *R* because a negative $[\alpha]_D^{20}$ was observed, which was in accord with that observed in cornoside ($[\alpha]_D^{20} -10.5^\circ$), and analysis of the ¹³C NMR data of C-1 and C-1''' in **3** indicated that they were greatly similar to that of C-1 (δ_C 68.9) in 4-[2-(β-D-glucopyranosyloxy)ethyl]-4-hydroxy-2-cyclohexen-1-one obtained from *Millingtonia hortensis* [19]. Consequently, the structure of **3** was determined as showed in Figure 1.

Marinoid I (**4**) was obtained as colourless oil. The presence of a molecular ion at *m/z* 701.2630 $[M + Na]^+$ (calcd $[M + Na]^+$, 701.2633) in the HRESIMS spectrum suggested a molecular formula of C₃₀H₄₆O₁₇. Analysis of the ¹H NMR data (Table 2) showed the presence of two cyclohexanone moieties in **4** [14] by the presence of the proton signals at δ_H 6.95 (1H, d, *J* = 10.0 Hz, H-2), 5.87 (1H, d, *J* = 10.0 Hz, H-3), 3.67 (1H, dd, *J* = 7.0, 3.6 Hz, H-6), 2.82 (1H, m, H-5α), and 2.49 (1H, m, H-5β)], and [δ_H 6.93 (1H, d, *J* = 10.0 Hz, H-2''), 5.85 (1H, d, *J* = 10.0 Hz, H-3''), 3.64 (1H, dd, *J* = 7.0, 3.6 Hz, H-6''), 2.82 (1H, m, H-5''α), and 2.49 (1H, m, H-5''β)], and the carbon signals at δ_C 198.6 (C-4), 154.7 (C-2), 127.1 (C-3), 72.4 (C-1), 82.8 (C-6), and 38.9 (C-5) and δ_C 198.6 (C-4''), 154.0 (C-2''), 127.0 (C-3''), 72.4 (C-1''), 82.7 (C-6''), and 38.9 (C-5''). The proton signals at δ_H 3.67 (1H, dd, *J* = 7.0, 3.6 Hz, H-6) and 3.64 (1H, dd, *J* = 7.0, 3.6 Hz, H-6''), and corresponding carbon signals at δ_C 82.8 (C-6), 82.7 (C-6''), 38.9 (C-5) and 38.9 (C-5'') revealed that **4** was the CH₃OH adduct of cornoside [13]. Moreover, the HMBC correlation from δ_H 3.43 (3H, s) to C-6 and δ_H 3.43 (3H, s) to C-6'' we therefore assign the methoxyl to C-6 and C-6'', respectively. NMR spectra also indicated the presence of two β-glucosyl groups, *i.e.*, two anomeric carbon resonances at δ_C 103.0 (C-1') and 102.8 (C-1''), and two anomeric protons at δ_H 4.28 (1H, d, *J* = 7.5 Hz, H-1') and 4.27 (1H, d, *J* = 7.5 Hz, H-1''). They were used as a starting point in the homonuclear correlated spectra to determine all the glycosidic protons. The $J_{H-1''-H-2''}$ value (7.5 Hz) and of $J_{H-1''''-H-2''''}$ value (7.5 Hz) compound **4**, further confirmed that the sugars were β-glucosyl groups [12]. The tentative molecular weight of the compound deduced from NMR analysis suggested compound **4** to be an unsymmetrical dimer of cornoside analog.

¹H-¹H COSY and HMBC correlations (Figure 2) were used to establish the molecular skeleton of **4**. Spin systems were revealed by analysis of COSY correlations corresponding to the H-2/H-3, H₂-5/H-6, H₂-1'/H₂-2', H-1''/H-2''/H-3''/H-4''/H-5''/H₂-6'', H-2'''/H-3''', H₂-5'''/H-6''', H₂-1''''/H₂-2''', and H-1''''/H-2''''/H-3''''/H-4''''/H-5''''/H₂-6'''. The connectivity of the two cyclohexanone moieties to C-1' and C-1''' were established by the HMBC correlations of H-2 to C-1' and H-6 to C-1', and of H-2''' to C-1''' and H-6''' to C-1''', respectively. The presence of the two β-glucosyl groups in **4** could be proposed on the basis of HMBC correlations from H-4'' to C-2' and H-1'''' to C-2'', respectively. The connection of the two β-glucosyl groups was confirmed by the presence of HMBC correlation from H-4'''' to C-1''.

The configuration of C-6, C-6''', C-1 and C-1''' were determined using the optical rotation and analysis of the coupling constants (J). The coupling patterns of the H-6 (dd, $J = 7.0, 3.6$ Hz) and H-5 α (dd, $J = 11, 3.6$ Hz); H-6''' (dd, $J = 7.0, 3.6$ Hz) and H-5''' α (dd, $J = 11, 3.6$ Hz) led to confirmation of the *cis*-orientation of H-6/H-5 α and H-6'''/H-5''' α [20,21], which are in line with those of 4-[2-(β -D-glucopyranosyloxy)ethyl]-4-hydroxy-5-methoxy-2-cyclohexen-1-one that was obtained from *M. hortensis* [19], and 1,6-dihydroxy-4-oxo-2-cyclohexene-1-cetic acid ethyl ester isolated from *Senecio scandens* [17], and thus the configuration of C-6 and C-6''' were determined as *S* and *S*. Analysis of the ^{13}C NMR data of C-1 and C-1''' in **4** indicated that they were greatly similar to that of C-1 (δ_{C} 71.8) in 4-[2-(β -D-glucopyranosyloxy)ethyl]-4-hydroxy-5-methoxy-2-cyclohexen-1-one [19], and our compound **4** showed $[\alpha]_{\text{D}}^{20}$ in MeOH of -26.4° , which is in accord with that observed in 1,6-dihydroxy-4-oxo-2-cyclohexene-1-acetic acid ethyl ester ($[\alpha]_{\text{D}}^{20} -12.5^\circ$) [17]. From the aforementioned analyses, the configurations of **4** were assumed to be 1*R*, 6*S*, 1'''*R* and 6'''*S*. On the basis of this cumulative analysis, the structure of **4** was thus determined as shown in Figure 1.

The cellular antioxidant assay (CAA) is a new approach to quantify antioxidants under physiological conditions when compared to chemical antioxidant activity assays [22–24]. The CAA assay has been widely used for fruits and vegetables recently, but not yet in marine natural products research. The EC_{50} values of compounds **1–3** were weak, 598, 4971, and 1103 μM , respectively. However, the EC_{50} value of compound **4** was 26 μM , of the same order of the positive control quercetin ($\text{EC}_{50} = 11 \mu\text{M}$).

3. Experimental Section

3.1. General Experimental Procedures

UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer (Wellesley, MA, USA). The IR spectra were measured in KBr on a WQF-410 FT-IR spectrophotometer (Beifen-Ruili, Beijing, China). NMR spectra were recorded on a Bruker AV 600 NMR spectrometer (Bruker, Bremen, Germany) with TMS as an internal standard. HR-ESI-MS data were obtained from Bruker Maxis mass spectrometer (Bruker, Bremen, Germany). Waters-2695 HPLC system (Waters, Milford, MA, USA), using a SunfireTM C₁₈ column (150 \times 10 mm i.d., 10 μm , Waters, Milford, MA, USA) coupled to a Waters 2998 photodiode array detector (Waters, Milford, MA, USA). Optical rotation data were measured by Perkin-Elmer Model 341 polarimeter (Wellesley, MA, USA). CD spectra were recorded on a spectropolarimeter (MODEL J-810-150S, Tokyo, Japan). The silica gel GF₂₅₄ used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, China. Spots were detected on TLC under UV light or by heating after spraying with 5% H₂SO₄ in EtOH. All solvent ratios are measured v/v.

3.2. Plant Material

The fruits of *A. marina* were collected from Beihai city, Guangxi province, China, in September, 2011. The specimen was identified by Professor Hangqing Fan who is from Guangxi Mangrove Research Center, Guangxi Academy of Sciences. A voucher specimen (2011-GXAS-008) was

deposited in Guangxi Key Laboratory of Marine Environmental Science, Guangxi Academy of Sciences, China.

3.3. Extraction and Isolation

The fruits of *A. marina* (35.4 kg, wet weight) were exhaustively extracted with EtOH-CH₂Cl₂ (2:1, v/v). The solvent was evaporated *in vacuo* to afford a syrupy residue that was suspended in distilled water and fractionated successively with petroleum ether, ethyl acetate, and *n*-butanol. The *n*-butanol soluble portion (269 g) was subjected to column chromatography (CC) on silica gel, using CHCl₃-MeOH (from 10:0 to 0:10) as eluent, giving eleven fractions (A–K). Fraction D was subjected to column chromatography to afford four subfractions (D1–D4). Fraction D3 was separated by HPLC, using MeOH-H₂O (MeOH:H₂O = 15:85, 25:75, 60:40) to yield **4** (3.9 mg, *R*_t = 10.2 min), **1** (3.5 mg, *R*_t = 12.5 min) and **2** (2.3 mg, *R*_t = 13.9 min), respectively. Fraction D4 was separated by HPLC, using MeOH-H₂O (MeOH:H₂O = 5:95) to yield **3** (6.0 mg, *R*_t = 9.5 min).

Marinoid F (**1**): Yellow oil; UV (MeOH) λ_{\max} (log ϵ_{\max}) 220 (2.45) and 242 (3.31) nm. $[\alpha]_D^{20}$ −14.7° (c 0.18, MeOH); CD (MeOH) $\Delta\epsilon_{220\text{ nm}}$ −4.28, IR (KBr) ν_{\max} 3425, 1720 and 1682 cm^{−1}. ¹H (CD₃OD, 600 MHz) and ¹³C (CD₃OD, 150 MHz) NMR data, see Table 1; HRESIMS: *m/z* 351.0835 (calcd. for C₁₄H₁₉ClO₈ + H, 351.0841).

Marinoid G (**2**): Yellow oil; UV (MeOH) λ_{\max} (log ϵ_{\max}) 220 (2.39) and 240 (2.75) nm. $[\alpha]_D^{20}$ +10.2° (c 0.21, MeOH); CD (MeOH) $\Delta\epsilon_{220\text{ nm}}$ +5.97; IR (KBr) ν_{\max} 3424, 1711 and 1684 cm^{−1}. ¹H (CD₃OD, 600 MHz) and ¹³C (CD₃OD, 150 MHz) NMR data, see Table 1; HRESIMS: *m/z* 351.0837 (calcd. for C₁₄H₁₉ClO₈ + H, 351.0841).

Marinoid H (**3**): Colourless oil; UV (MeOH) λ_{\max} (log ϵ_{\max}) 218 (2.35) and 237 (2.94) nm. $[\alpha]_D^{20}$ −15.8° (c 0.25, MeOH); CD (MeOH) $\Delta\epsilon_{220\text{ nm}}$ −6.32; IR (KBr) ν_{\max} 3452, 1701 and 1675 cm^{−1}. ¹H (CD₃OD, 600 MHz) and ¹³C (CD₃OD, 150 MHz) NMR data, see Table 1; HRESIMS: *m/z* 591.2281 (calcd. for C₂₆H₃₈IO₁₅ + H, 591.2283).

Marinoid I (**4**): Colourless oil; UV (MeOH) λ_{\max} (log ϵ_{\max}) 219 (2.15) and 238 (3.05) nm. $[\alpha]_D^{20}$ −26.4° (c 0.31, MeOH); CD (MeOH) $\Delta\epsilon_{220\text{ nm}}$ −5.42; IR (KBr) ν_{\max} 3447, 1705 and 1679 cm^{−1}. ¹H (CD₃OD, 600 MHz) and ¹³C (CD₃OD, 150 MHz) NMR data, see Table 1; HRESIMS: *m/z* 701.2630 (calcd. for C₃₀H₄₆O₁₇ + Na, 701.2633).

3.4. Cellular Antioxidant Assay

Following the reported method [21–23], the cellular antioxidant activity was determined.

4. Conclusions

In conclusion, four new jacaranone analogs, marinoids F–I (**1–4** respectively), were isolated from a Beibu Gulf mangrove *A. marina* and identified. Marinoids F and G are shown to be diastereoisomers of chlorocornoside, a new halogen containing marine secondary metabolite. The CAA assay is considered to be a more physiologically relevant assay in the measurement of antioxidant activity of

food when compared to the common chemistry antioxidant activity assays [25,26]. Until today, there have been no reports of the use of this CAA assay in the marine research area. Using the assay, the antioxidant activity of the isolates was therefore determined. This is the first report of chlorocornoside and of the dimeric disaccharide **4** which showed good antioxidant activity ($EC_{50} = 26 \mu M$), comparable with the positive control quercetin.

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Author Contributions

In this paper, Xiang-Xi Yi was in charge of writing the manuscript; Yong Chen and Ming-Ben Xu was responsible for the isolation of the compounds; Yin-Ning Chen was responsible for structures identification; Wen-Pei Xie was in charge of biological activity; Cheng-Hai Gao is the corresponding author who was responsible for the analysis of the data of biological activity; and Ri-Ming Huang is the corresponding author who was responsible for arranging, checking and revising the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Sun, Y.; Ouyang, J.; Deng, Z.W.; Li, Q.S.; Lin, W.H. Structure elucidation of five new iridoid glucosides from the leaves of *Avicennia marina*. *Magn. Reson. Chem.* **2008**, *46*, 638–642.
2. Fauvel, M.T.; Taoubi, K.; Gleye, J.; Fourasté, I. Phenylpropanoid glycosides from *Avicennia marina*. *Planta Med.* **1993**, *59*, 387.
3. König, G.; Rimpler, H. Iridoid glucosides in *Avicennia marina*. *Phytochemistry* **1985**, *24*, 1245–1248.
4. Feng, Y.; Li, X.M.; Duan, X.J.; Wang, B.G. Iridoid glucosides and flavones from the aerial parts of *Avicennia marina*. *Chem. Biodivers.* **2006**, *3*, 799–806.
5. Fauvel, M.T.; Bousquetmelou, A.; Moulis, C.; Gleye, J.; Jensen, S.R. Iridoid glucosides from *Avicennia germinans*. *Phytochemistry* **1995**, *38*, 893–894.
6. Ito, C.; Katsuno, S.; Kondo, Y.; Tan, H.T.W.; Furukawa, H. Chemical constituents of *Avicennia alba*. Isolation and structural elucidation of new naphthoquinones and their analogues. *Chem. Pharm. Bull.* **2000**, *48*, 339–343.
7. Han, L.; Huang, X.S.; Dahse, H.M.; Moellmann, U.; Fu, H.Z.; Grabley, S.; Sattler, I.; Lin, W.H. Unusual naphthoquinone derivatives from the twigs of *Avicennia marina*. *J. Nat. Prod.* **2007**, *70*, 923–927.

8. Sharaf, M.; El-Ansari, M.A.; Saleh, N.A.M. New flavonoids from *Avicennia marina*. *Fitoterapia* **2000**, *71*, 274–277.
9. Han, L.; Huang, X.S.; Dahse, H.M.; Moellmann, U.; Grabley, S.; Lin, W.H.; Sattler, I. New abietane diterpenoids from the mangrove *Avicennia marina*. *Planta Med.* **2008**, *74*, 432–437.
10. Parker, K.A.; Kang, S.K. Regiospecific nucleophilic aromatic-substitution—Conjugate addition of active methylene-compounds to quinone monoacetals and aromatization of the adducts. *J. Org. Chem.* **1980**, *45*, 1218–1224.
11. Hollenst, R.; Philipsb, W.V. Carbon magnetic-resonance spectra of α , β , γ , σ - and α , β , α' , β' -unsaturated-ketones. *Helv. Chim. Acta* **1972**, *55*, 2030–2044.
12. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH Publishers: New York, NY, USA, 1987; pp. 380–394.
13. Endo, K.; Hikino, H. Structures of rengyol, rengyoxide, and rengyolone, new cyclohexylethane derivatives from *Forsythia suspensa* fruits. *Can. J. Chem.* **1984**, *62*, 2011–2014.
14. Bianco, A.; Scalzo, R.L.; Scarpatti, M.L. Isolation of cornoside from *Olea europaea* and its transformation into halleridone. *Phytochemistry* **1993**, *32*, 445–457.
15. Jensen, S.R.; Kjaer, A.; Nielsen, B.J. A quinol glucoside isolated from *Cornus* species. *Acta Chem. Scand.* **1973**, *27*, 367–369.
16. Sasaki, H.; Taguchi, H.; Endo, T.; Yosioka, I. The glycosides of *Martynia louisiana* Mill. A new phenylpropanoid glycoside, martynoside. *Chem. Pharm. Bull.* **1978**, *26*, 2111–2121.
17. Tian, X.Y.; Wang, Y.H.; Yang, Q.Y.; Yu, S.S.; Fang, W.S. Jacaranone analogs from *Senecio scandens*. *J. Asian Nat. Prod. Res.* **2009**, *11*, 63–68.
18. Seo, S.; Tomita, Y.; Tori, K.; Yoshimura, Y. Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **1978**, *100*, 3331–3339.
19. Hase, T.; Kawamoto, Y.; Ohtani, K.; Kasai, R.; Yamasaki, K.; Picheansoonthon, C. Cyclohexylethanoids and related glucosides from *Millingtonia hortensis*. *Phytochemistry* **1995**, *39*, 235–241.
20. Huang, R.M.; Ma, W.; Dong, J.D.; Zhou, X.F.; Xu, T.H.; Lee, K.J.; Yang, X.W.; Xu, S.H.; Liu, Y.H. A new 1,4-diazepine from South China Sea marine sponge *Callyspongia* species. *Molecules* **2010**, *15*, 871–877.
21. Wang, Y.L.; Li, Z.L.; Zhang, H.L.; Sha, Y.; Pei, Y.H.; Hua, H.M. New germacrane sesquiterpenes from *Salvia chinensis*. *Chem. Pharm. Bull.* **2008**, *61*, 843–846.
22. Faller, A.L.K.; Fialho, E.; Liu, R.H. Cellular antioxidant activity of Feijoadá whole meal coupled with an *in vitro* digestion. *J. Agric. Food Chem.* **2012**, *60*, 4826–4832.
23. Song, W.; Derito, C.M.; Liu, M.K.; He, X.J.; Dong, M.; Liu, R.H. Cellular antioxidant activity of common vegetables. *J. Agric. Food Chem.* **2010**, *58*, 6621–6629.
24. Wolfe, K.L.; Kang, X.M.; He, X.J.; Dong, M.; Zhang, Q.Y.; Liu, R.H. Cellular antioxidant activity of common fruits. *J. Agric. Food Chem.* **2008**, *56*, 8418–8426.
25. Wolfe, K.L.; Liu, R.H. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *J. Agric. Food Chem.* **2007**, *55*, 8896–8907.

26. Felice, D.L.; Sun, J.; Liu, R.H. A modified methylene blue assay for accurate cell counting. *J. Funct. Foods* **2009**, *1*, 109–118.

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